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# THE CYTOLOGY OF EOCRONARTIUM MUSCICOLA

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The view of Brefeld (5) that the promycelium of the Uredinales and Ustilaginales is homologous with the transversely septate basidium of the Auriculariales has received general acceptance. His theory, based on a morphological study of the two structures, has been substantiated by the results of subsequent cytological investigations on members of these three groups and of the higher Basidiomycetes. Consequently the Auriculariales are regarded as closely related to the Uredinales, and as probably intermediate in origin between them and the higher Hymenomycetes. Comparatively little is known, however, concerning the nuclear history and general cytology of members of this order. Only species of the genus Auricularia have been examined, and here the facts are only partially determined. The investigation of members of other genera is therefore desirable, and should shed further light on the phylogeny of the Basidiomycetes.

A preliminary examination of stained sections of the sporophore of *Eocronartium muscicola* (Fries) Fitzpatrick, made in connection with the writer's (12) study of the parasitism of this species, disclosed the fact that unusually large nuclei make this form a favorable subject for cytological investigation. The present paper is an outgrowth of this discovery, and embodies the results of an investigation of the cytology of this species conducted during the past three years.

## MATERIALS AND METHODS

*Eocronartium muscicola* is an obligate parasite occurring on a considerable number of mosses of various genera. It produces small Typhula-like sporophores at the apices of the branches of the moss gametophore, in which it exists as a perennial, intracellular parasite.

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A detailed account of its parasitism, life history, and morphology has already been given in the writer's previous paper (12), and the facts need not be repeated here.

The present investigation of the cytology of the species is based on material taken from a single host, *Climacium americanum* Brid., all the collections having been made by the writer in the vicinity of Ithaca, N. Y. *Climacium americanum* is a large moss, and the fungous sporophores produced on its branches are larger than those developed on smaller species. They are consequently more favorable for study. The large size of the host, moreover, renders it especially suitable in connection with the staining of the endophytic mycelium. Fortunately the fungus has been collected more abundantly on this host than on any other.

Under favorable weather conditions the young sporophores, which make their appearance at the tips of the gametophoric branches, undergo rapid elongation, and within a period of two weeks begin to bear basidia and form spores. If dry weather prevails their development is retarded, and material favorable for cytological examination is not easily procured in the field. Parasitized host plants were, therefore, collected in the early summer, and placed in the greenhouse under conditions favorable for growth. Abundant moisture was provided and normal fungous sporophores developed in great numbers. In this manner excellent material illustrating all stages in the development of the fruit-body and its hymenium was easily obtained.

Sporophores intended for subsequent cytological study were removed from the host at varying intervals and given a preliminary microscopic examination before they were placed in the fixing solution. A brief examination of the individual sporophores under the lower powers of the microscope proves of considerable service in demonstrating the presence of spores. Their comparative abundance furnishes a criterion for the determination of the age of the basidia. Since basidia of practically all stages of development are commonly found together on a single sporophore, this preliminary examination gives, however, only an indication of the predominating stages present in the hymenium. Sporophores collected in the field at different times during the growing season were placed in the fixing solution and subsequently studied in comparison with those developed in the greenhouse.

For study of the endophytic mycelium, diseased host plants were

collected at all seasons of the year. Portions of the main axes of the gametophores and their branches as well as pieces of the procumbent "stolon" were removed from the plant and placed in fixing solution. Material was selected from the newer light green branches and from the dark green parts developed the previous year. A careful examination of an infected individual shows that the hyphae are present in both the new and old growths. Healthy host plants were also collected, and portions of these were used for comparison.

The most satisfactory fixing agent used was the medium strength solution of chromo-acetic acid recommended by Chamberlain (6). It gave wholly satisfactory fixation, while solutions containing osmic acid proved less useful. Air was removed from the material by means of a suction pump. This is particularly necessary when pieces of the host plant are treated, since the branches of the gametophore are sheathed by small imbricated leaves, and the stolons are covered with numerous closely matted rhizoids. The majority of the sections were cut 5$\mu$ in thickness; none exceeded 7$\mu$. They were for the most part cut parallel to the long axis of the sporophore or gametophoric branch, few transverse sections being made.

Various combinations of stains were used. Heidenhain's iron alum-haematoxylin was given a thorough trial but proved unsatisfactory. It was used alone and in combination with certain other stains such as Congo red, fuchsin, and erythrosin. Small cytoplasmic granules stain deeply with haematoxylin, and obscure the details of the nuclear structure. The triple stain of Flemming gave better results. After a few trials it became evident that in the study of *Eocronartium muscicola* the use of the shortened method recommended by Harper and others is far superior to the longer schedules previously employed. A sharper differentiation of the chromatin and achromatic structures, fibers, centrosomes, and nucleoli is obtained. This fact combined with the economy of time resulting from its use makes the shorter method much more advantageous. The three stains in the combination were applied for various periods of time, the following schedule proving most satisfactory: safranin 1 minute, gentian violet 5–15 minutes, orange G 10–20 seconds.

The nuclei in the various structures of the fungus seem to possess an almost equal affinity for the stains, while the cytoplasm in the basidia, spores, and hyphae is affected relatively little. The cytoplasm of the host cells stains slowly and does not obscure the hyphae appreciably, but the host nuclei stain sharply and rapidly.

The spores of this fungus can be induced to germinate very readily in tap water or synthetic solutions practically 100 percent germination being obtained in twenty-four hours. The germinated spores were stained and permanent mounts of these made, the following method having been used.

A drop of sterilized water was placed in the center of a sterilized glass slide, and a sporophore of the fungus was washed in this. Since the spores fall away easily, this procedure resulted in a spore suspension containing hundreds of spores. After a microscopic examination to determine whether a sufficient number of spores were present, this drop was drawn up into a small pipette. Several clean cover glasses were then provided, and a small drop of this spore suspension was transferred from the pipette to the center of each. These cover glasses were then inverted over van Tieghem cells. The hanging drop culture which was thus obtained could be examined frequently under the microscope, and the germination of the spores watched.

When spore germination had progressed to the desired point the cover glass was carefully removed from the cell, and the spore suspension subjected to the fumes of osmic acid. The stopper was removed from a narrow-mouthed bottle containing a strong solution of the acid, and the cover glass was placed over the opening so that the drop hung inside enveloped in the fumes. Several minutes of this treatment were sufficient to effect fixation. In some cases this drop was then stippled upon a slide smeared with albumin fixative, the method recommended by Harper (17) being followed from this point. In other cases it was allowed to dry on the cover glass. The procedure, recommended by Harper, of diluting the suspension of germinated spores with a drop of Flemming's fixing solution, results in a scattering of the spores and gave less desirable preparations. After the spores had dried down on the cover glass or slide they were carried through the usual staining schedule.

## Endophytic Mycelium

The hyphae of *Eocronartium muscicola* in the tissues of the host (figs. 1–4) are more irregular in shape and more frequently branched than those composing the sporophore. They vary considerably also in size, the threads in the larger cells of the basal portions of the gametophore being in general of larger diameter than those in the smaller embryonic cells of the apical point or those in the cells of the leaves.

Great variation is shown also in the length of the individual cells of the mycelium, some cells being many times longer than others (figs. 1, 2). The transverse septa are sharply defined. In stained material they cannot be easily overlooked. At or near the center of each septum there is usually present on one or both surfaces a large, hemispherical or disc-like, deep-staining body (figs. 1–4). These structures also occur on the septa of the hyphae making up the sporophore (figs. 5, 6, 14, 16). They stain deeply with gentian violet and Heidenhain's iron alum-haematoxylin. It is interesting to note that Levine (32), who found what appear to be identical structures in several species of Boletus, states that they stain a deep red with safranin.

These disc-like structures are described as of common occurrence on the cross walls of hyphae in many Basidiomycetes and certain Ascomycetes, and are frequently figured. They were apparently first mentioned by Hoffmann (22). Strasburger (49) found them in *Agaricus campestris* and states that they mark the position of intercellular pores, thus indicating protoplasmic continuity. They have subsequently been found by Rosenvinge (46) in *Clavaria vermicularis*, by Ruhland (47) in *Lepiota lilacino-granulosa*, by Harper (19) and Nichols (38) in *Coprinus ephemerus*, by Levine (32) in *Polystictus versicolor*, *Polyporus adustus*, *Polyporus betulinus*, *Polyporus destructor*, *Boletus granulatus*, and *Coniophora cerebella*, and by Kniep (27, 28) in *Coprinus nycthemerus*, *Corticium varians*, *Corticium serum*, and *Polyporus destructor*. They have also been described by Harper (18) in *Pyronema confluens*, and by the writer (11) in *Rhizina undulata*. They are apparently common in many of the higher fungi, but in certain species are stated definitely to be absent. Their occurrence is not associated especially with either clamp connections or hyphal anastomoses. The specific function of these bodies is doubtful, and no attempt has been made by the writer to determine their exact nature. The difference between the writer's results and those of Levine with reference to their affinity for stains indicates, however, that their composition is not uniformly the same.

The cells of the endophytic hyphae in *Eocronartium muscicola*, in all the cases observed by the writer, are clearly binucleate (figs. 1–4). A painstaking search through many slides has failed to reveal uninucleate or multinucleate cells. Since the transverse septa in stained material are sharply defined, the number of nuclei in a given cell cannot be easily mistaken. As is to be expected, occasional cells contain-

ing four nuclei are found. The position of the nuclei in the cell in such a case indicates, however, that conjugate division has recently taken place, the intervening septum having not yet formed. The rarity of these four-nucleate cells shows that the septum is laid down rapidly immediately after the completion of the mitosis. The hyphae in all parts of the host from the stolons to the tips of the branches have been given critical examination, and in all the material studied the cells are undoubtedly binucleate throughout. Branches from infected plants have been placed in the fixing solution at all seasons of the year, but no variation from the binucleate condition has been observed. If uninucleate or multinucleate cells occur in the endophytic mycelium, they must be present in early stages immediately following infection. No material showing these early infection stages has been collected. The failure of the writer (12) to obtain infection has, consequently, rendered impossible the determination of the point of origin of the binucleate condition.

The two nuclei in any given cell are of practically the same size. Those in different hyphae, and in different cells of the same hypha, vary considerably in diameter. None of the nuclei in the endophytic hyphae reach the large dimensions of certain of those in the interior of the sporophore. They are, therefore, less favorable for study of conjugate division. In fact, none of the details of mitosis have been clearly observed in the hyphae in the host cells.

## The Sporophore

The binucleate condition is maintained in all the cells of the hyphae composing the sporophore (figs. 5–16). Uninucleate or multinucleate cells have never been observed. The nuclear pairs divide in all cases by conjugate division, and the daughter nuclei are soon separated by the formation of transverse septa. The two nuclei of any pair are of practically the same diameter, but great difference in size is evident between nuclei of different cells (figs. 5, 6). The greatest variation also exists in the diameter of the hyphae and in the length of the cells. In general, the hyphae lying near the center of the sporophore are of larger diameter than those near the periphery.

The variation in size of the nuclei in the cells of the sporophore is remarkable. Some of these reach a diameter nearly as great as that of the fusion nucleus of the basidium, while others in adjacent hyphae are more minute than the nuclei of the spores. It is difficult to explain

this great difference, though two factors are evidently involved. The large amount of cytoplasm in the larger cells probably necessitates a corresponding increase in the mass of the nuclei. Also it is evident that the nuclei increase greatly in size as they pass into mitosis, the largest nuclei observed being in process of division.

The two nuclei of a cell, when in the resting condition, usually lie a considerable distance apart, frequently in opposite ends of the cell. This is especially true of extremely long cells. When preparing to divide, the nuclei migrate toward the center of the cell, approach each other, and often come to lie in actual contact. In the majority of cases nuclei at this stage are of so large a diameter that it is impossible for them to pass each other in the thread, or to assume the side-by-side position typical of conjugate division. At all stages the nucleolus is evident as a spherical homogeneous body, staining deeply with safranin, and easily distinguishable from the chromatin material in the nucleus. The chromatin stains sharply, and in stages preceding the formation of the spindles is frequently contracted into a compact mass in one side of the nucleus (figs. 6, 7), the nucleolus occupying the other. In poorly stained preparations a pair of nuclei in this condition have the appearance of four small nuclei. When well stained the nuclear membranes are evident, and the two deep-staining bodies in each nucleus then are seen to lie in a common, hyaline nuclear cavity.

The two nuclei pass from the resting condition into mitosis together. The formation of the spindles is not, however, always exactly simultaneous (figs. 9–14). At all stages up to late anaphase each nucleus possesses a well defined membrane, and the spindle, which is intranuclear, stands out sharply in the nuclear cavity accompanied by the nucleolus. The spindles are not necessarily parallel. Moreover they lie without any reference to the long axis of the cell.

The spindle fibers stain clearly, and there is visible in some cases at each pole of the spindle a more deeply staining point. This is doubtless the centrosome, but its minuteness precludes any study of its structure. In certain cases it has the appearance of a short rod resembling that described and figured by Harper (20) in Phyllactinia. The writer has never seen any indication of astral rays. The preparations examined have proved very favorable for a study of division stages. Equatorial plate stages are especially numerous, and in many of these cases it is possible to count the chromosomes with a reasonable degree of certainty. All the evidence accumulated shows the number

to be four. Occasionally also four chromosome-like bodies can be seen in a nucleus which shows no indication of a spindle, and which is evidently in an early prophase (fig. 8).

When the chromosomes begin to pass toward the poles the nuclear membrane is still evident, but it disappears soon afterward. In figure 15 the membrane is absent, and the chromosomes are shown scattered over the spindle. Here also the chromosome number is clearly shown to be four. The two spindles orient themselves parallel to the long axis of the cell, and soon come to lie side by side in typical conjugate division (fig. 16). The nucleolus is drawn into the spindle and passes toward one of the poles. In late telophase it is incorporated in one of the daughter nuclei.

Although in metaphase and early anaphase the spindles do not occupy the position characteristic of conjugate division, they pass more or less definitely into this position before late telophase, and it is evident that the two daughter nuclei which migrate into each end of the cell are not in any case sisters. At the completion of the division the four resulting nuclei round up and remain for a brief period as well defined nuclei in a single cell (figs. 17, 18). The fact that these four-nucleate cells are rarely found indicates that the transverse septum is formed quickly. After the laying down of the septum the two resulting cells elongate, and the nuclei in each drift apart.

As the sporophore approaches maturity, the hyphae at its periphery undergo a slight amount of branching, the terminal portions turning out at right angles to the long axis of the sporophore. A more or less definite palisade layer is thus formed. The terminal cells later undergo further growth, and develop into basidia. The basidia do not stand close together, and a definite hymenium, such as occurs in most of the higher Basidiomycetes, is not formed. Paraphyses are absent, and no sharp differentiation of the fruit-body into subhymenium and trama occurs. The hyphae composing the sporophore interweave only to a slight degree, and a loose tissue results in which an individual hypha may be traced from the basidium far back into the fruit-body.

## Nuclear Phenomena in the Basidium

When the basidium is merely the undifferentiated terminal cell of one of the hyphae composing the sporophore it contains two small nuclei similar to those present in other cells of the thread. As it

enlarges these nuclei increase rapidly in size (figs. 20–23). They also become more sharply staining, and migrate toward the center of the cell. Here they soon come into actual contact and finally fuse (fig. 24), the membranes of the two nuclei being dissolved at the point of contact and a common cavity resulting. The chromatin strands of the two nuclei intermingle completely even before the deep constriction about the common nuclear cavity has disappeared. The resulting fusion nucleus soon rounds up, and for a time two nucleoli are present (fig. 25). The absence of any evidence of disintegration in these nucleoli, combined with the fact that in later stages the fusion nucleus contains a single large nucleolus, indicates that the two bodies soon fuse.

The fusion nucleus assumes at once the resting condition (fig. 26), a delicate reticulum being formed. It undergoes also a pronounced increase in size, and in this and subsequent stages stains sharply. As it prepares to pass from the resting condition into mitosis, the chromatin granules fuse to form larger masses at the interstices of the network (fig. 27). These larger masses then gradually take on an elongated shape, and a definite thread is formed. This is thrown into definitely thickened loops which appear to be eight in number (fig. 28). Since the diploid chromosome number is eight, these loops probably represent the chromosomes. The spirem condition (fig. 29) which thus results is striking in appearance, and the large number of nuclei at this stage in the preparations show it to be of relatively long duration.

The spirem gradually passes to one side of the nucleus (figs. 30, 31), and finally contracts into a typical synaptic knot (fig. 32). In some cases the nucleolus is caught in this (figs. 31, 32), in others it lies free in the other half of the nucleus (fig. 30). The nucleolus shown in figure 32 stands out clearly as a red sphere within an enveloping tangle of blue chromatin. In figure 33 a nucleus is shown in which the spirem has apparently undergone longitudinal splitting. Nuclei presenting this appearance are uncommon in the preparations, and the writer is in doubt concerning the point. In subsequent stages the spirem segments (fig. 34), and the segments shorten into chromosomes. The nucleolus persists throughout all stages of mitosis, and passes into one of the daughter nuclei in late telophase.

The spindle (figs. 35, 36) resembles in shape and general appearance those which are formed in the conjugate divisions in the hyphae of the sporophore but is larger. The achromatic fibers form a well defined

bipolar spindle, and at each pole the centrosome appears as a deeply staining point (figs. 35, 36). Astral radiations have not been observed. The chromosomes are somewhat elongated, rod-like bodies, and when they occupy the equatorial region can be counted with certainty. They do not scatter as they migrate toward the poles as in the conjugate divisions, but are drawn apart in two well defined groups. The spindle at the beginning of the division is definitely intranuclear, but the nuclear membrane soon breaks down, and at late telophase the two groups of chromosomes lie free in the cytoplasm with indications of spindle fibers between them (fig. 37).

The daughter nuclei soon round up, assume a definite membrane, and a nucleolus appears in each (fig. 38). At the completion of the mitosis the basidium undergoes rapid elongation, and the two nuclei migrate apart. The basidium soon attains its full length, and the nuclei enter the second mitosis.

Few basidia have been found undergoing the second nuclear division, and it probably consumes far less time than the first. The spindle in this second mitosis is smaller than that in the first but resembles it in all other respects (fig. 39). The chromosome number is clearly four. The divisions in the two nuclei are not necessarily exactly simultaneous. In figure 39 the upper nucleus shows the two groups of chromosomes passing toward the poles, while in the lower nucleus the separation has not yet occurred.

Before the completion of the second division a transverse septum begins to form near the center of the basidium, and when the daughter nuclei have rounded up the basidium is composed of two binucleate cells (fig. 40). When one of the nuclei resulting from the first mitosis divides more rapidly than the other, a second septum may be laid down in one of these cells before it appears in the other (fig. 41). Usually, however, these two septa are formed simultaneously so that the typical four-celled basidium results (fig. 42). Maire (36) figures and describes in *Auricularia mesenterica* similar cases in which the two septa last formed are laid down independently because of the more rapid division of one of the two nuclei.

Comparatively few basidia in the preparations show stages following the spirem condition of the fusion nucleus, and preceding the four-celled basidium. It is evident that the mitoses are completed and the septa laid down in a relatively short space of time. Four-celled basidia which have not yet begun to form sterigmata are numerous in

many of the sections. The nuclei in the four-celled basidium are all of the same size, and are considerably smaller than the fusion nucleus. The size varies little in different basidia and is maintained in the spore.

## Sterigmata and Spore Formation

In the young condition the basidia stand at right angles to the surface of the sporophore and form a more or less definite palisade layer. During their elongation they fall over and assume a procumbent position. An examination of mature basidia shows that the resulting bend usually takes place in the basal cell of the basidium (figs. 42–45) rather than in the hypha which bears it. This procumbent position results naturally from the lack of rigidity in the long, slender, flexuous basidium, but is nevertheless of decided importance in that it allows the sterigmata to arise at right angles to the surface of the sporophore unhindered by contact with neighboring basidia or sterigmata. The sterigmata are developed consequently in a palisade layer almost as well marked as that of the young basidia. In fact, when both young and old basidia lie close together, as is commonly the case, the palisade may be composed of a mixture of these two structures.

Each cell of the basidium gives rise to a single long, cylindrical, flexuous sterigma (figs. 44, 45), which bears at its tip an elongated, more or less crescent-shaped spore. The sterigma frequently reaches a length two-thirds that of the mature basidium, and has a diameter approximately the same as that of its nuclei. In rare cases sterigmata may arise simultaneously from all the cells, but far more frequently they originate independently of one another. In some cases the apical cell is the first to bud, in others it is the last. As the sterigma pushes outward, the cytoplasm in the cell behind becomes increasingly vacuolate, and finally the basidium is entirely emptied. The nuclei in the various cells pass outward with the cytoplasm into their respective sterigmata. In some cases the nucleus passes out relatively early, in other cases it remains in the basidium until a sterigma of considerable length has formed. Before its passage outward it is globose, but in the tube it becomes somewhat elongated. This elongation is probably due in large measure to the stress exerted upon the membrane by the flowing cytoplasm. It is not due to the narrowness of the sterigma, since in some cases, in which marked elongation occurs, the diameter of the sterigma exceeds even the long diameter of the nucleus.

As the sterigma pushes outward, its tip is broad and rounded (figs. 43–45), but on reaching its full length it becomes acuminate (fig. 46). The production of a minute globose body at the tip marks the beginning of spore formation (fig. 47). This structure, termed the spore "initial" by Levine (32), gradually increases in size and elongates, the cytoplasm of the sterigma flowing out into it through the very narrow canal which results (figs. 48–56). While the young spore is forming at the tip of the sterigma the nucleus lies remote from this point. There is no evidence to show that it influences directly the formation of the spore "initial." As the cytoplasm flows outward the nucleus is carried along with it, and on reaching the narrow canal at the tip of the sterigma becomes greatly elongated, the diameter of the normal nucleus being many times that of the canal. The nucleus is, in fact, drawn out into a long rod, and all trace of the nuclear membrane is lost (figs. 51–54). The nuclear material at this stage stains deeply, and the rod has an irregularly beaded appearance. The size of the spore at the time of the entrance of the nucleus varies, but it has in most cases reached at least half its mature length. The nucleus after its entrance into the spore remains in some cases for a long time in the rod-like condition. Finally it contracts into an irregularly globose, homogeneous, deep-staining mass (fig. 55), which soon takes on a nuclear membrane and assumes the characters of a normal globose, resting nucleus (fig. 56). The development of the narrow canal and the assumption by the nucleus of the irregular rod-like form recall similar phenomena described by Levine (32), Maire (36), Petri (44), Fries (13), and other workers for various higher Basidiomycetes. There is no reason to believe, however, that in *Eocronartium muscicola*, as in some of these cases, the centrosome is involved in forming the sterigma or in directing the course of the nucleus through the canal into the spore. The evident fibrillar strands which Levine (32) describes in Boletus as extending from the centrosome in the spore "initial" down through the canal to the definitely beaked nucleus in the basidium have no counterpart in Eocronartium. The passage of the nucleus into the sterigma is accomplished simply by the outward flow of the cytoplasm, and its passage through the canal into the spore is evidently of similar nature, no pull by kinoplasmic strands being exerted. In a unicellular basidium, as in Boletus, there is evidently need of a specialized apparatus for directing the course of the different nuclei into their respective sterigmata, since without such a

controlling apparatus one spore might receive two or more nuclei and another none. The formation of septa in *Eocronartium muscicola* eliminates this possibility.

Since a large number of nuclei in the rod-like condition are present in the preparations, it is evident that considerable time is consumed in the passage from the sterigma into the spore. This results from the great length of the nucleus, and probably also from the fact that the denser nature of the nucleus retards its flow through the canal.

After the passage of all the cytoplasm into the spore (fig. 57), the spore is freed from the sterigma. The writer has not studied the phenomena attending the liberation of the spores, and cannot say whether or not they are forcibly discharged as in many other Basidiomycetes. The mature spore contains a single globose nucleus. A binucleate spore has never been observed.

## Spore Germination

In wet weather spores frequently begin to germinate on the sporophore where they have fallen among the basidia (fig. 59). They can be induced to germinate very readily in the laboratory in tap-water or in synthetic nutrient solutions (figs. 60, 61). Germination takes place by the formation of one or more germ-tubes, and the spore germinates in the uninucleate condition. The division of this nucleus has not been observed, and germ-tubes containing more than one nucleus have not been found. The writer in his previous paper (12) on *Eocronartium muscicola* discusses in detail the phenomena exhibited in spore germination and figures all the stages obtained. The reader is referred to the sections of this paper on spore germination and inoculation experiments for a complete discussion of the problems encountered in the attempts to obtain later stages in spore germination. The failure to obtain these has rendered impossible the explanation of the origin of the binucleate condition of the mycelium in this species. Since the spore germinates in the uninucleate condition, and all the cells of the endophytic mycelium and sporophore ever observed are binucleate, it is probable that the binucleate series of cells arises in the germ-tube soon after germination, but they have not been observed. No clamp connections have been found either on the endophytic hyphae or in the sporophore. It is not impossible that they may be produced for a brief period on the young mycelium, but their absence on older threads renders such a supposition extremely doubtful. Consequently the re-

cent explanation of the maintenance of the binucleate condition in the Basidiomycetes advanced by Kniep (28) cannot be applied to *Eocronartium muscicola*. The conjugate divisions in this species certainly take place without the assistance of clamp connections. Moreover, clamp connections are never found on the basidia.

## General Considerations

The discovery in the Uredinales of sexual cell fusions accompanied by a well defined alternation of generations leaves no room for doubt that in this group of the Basidiomycetes sexuality exists. The now familiar observations of Blackman (2, 3) and Christman (7, 8, 9) have been confirmed and amplified by investigations by Olive (39, 40, 41, 42, 43), Kurssanow (31), Hoffmann (21), Arnaud (1), Fromme (15, 16), Kunkel (29, 30), and others on various species and on special phases of the cytology of the group. The mass of evidence accumulated demonstrates that in the Uredinales a generation of uninucleate cells alternates with a generation of binucleate ones, the binucleate series arising by the fusion of two uninucleate cells, and the nuclear fusion which occurs universally in the mature teleutospore being followed in the promycelium by what is with reasonable certainty a numerical reduction of the chromosomes. Proof of conjugate divisions in the hyphae and in spore formation is undoubted. The positive results obtained have stimulated research on species in other orders of the Basidiomycetes. Comparatively little is known, however, of the closely related group, the Auriculariales.

Istvanffi (24) describes the germination of the basidiospores in *Auricularia Sambucina*, and figures a single spore transversely septate into two uninucleate cells, each giving rise to a cluster of curved, uninucleate conidia. He gives no other figures, and makes no further contribution to the cytology of the group.

Sappin-Trouffy (48), working with *Auricularia auricula-judae*, traces the nuclear history from the young basidium to the mature spore. He states that the fruit-body is composed of interwoven hyphae possessing frequent transverse septa, the cells, in many cases at least, being binucleate. He makes no effort to determine the point of origin of this binucleate condition, and fails to state definitely whether multinucleate or uninucleate cells occur. The basidia arise as terminal cells on the peripheral hyphae of the sporophore, and in the young condition are binucleate. The two nuclei in the basidium later fuse,

and the resulting fusion nucleus then increases rapidly in size. Later it divides, and the daughter nuclei migrate toward the ends of the now much elongated basidium. A transverse septum is then laid down. Subsequently these nuclei also divide and other septa are formed, the basidium being finally composed of four superimposed, uninucleate cells. From each cell a sterigma is then put out, and at its tip a spore begins to form. The spore after reaching maturity germinates in the uninucleate condition. In germination a secondary spore is developed, and the nucleus migrates into this. The germination of this secondary spore was not watched, and no later stages showing germ-tubes containing more than one nucleus were obtained. The nuclear divisions in the basidium were not actually observed, and no details of nuclear structures are figured or described. Branching, septate paraphyses composed of binucleate cells lie between the basidia. Sappin-Trouffy points out the resemblance between the transversely septate basidium of Auricularia and the internal promycelium of Coleosporium, but he lays little emphasis upon the point, and considers the basidium homologous with the oospore.

Juel (25), from the examination of another species, *Auricularia mesenterica*, gives a detailed account of the nuclear divisions in the basidium, but adds nothing to the knowledge of the nuclear history in this genus. He states that the fusion nucleus lies at the center of the cylindrical basidium and is of an elongated shape due to the narrowness of the cell. It contains an evident nucleolus and a delicate chromatin network. Without leaving its central position it undergoes mitosis, the nuclear membrane disappearing and the spindle lying parallel to the long axis of the basidium. Delicate astral rays may be seen at each pole radiating into the cytoplasm from a deeply staining point which seems to be a centrosome. On the spindle there are six or eight deep-staining bodies which Juel regards as chromosomes. In the second division in the basidium the spindles are smaller and stouter, and lie within a well defined nuclear membrane. They are in all other respects similar to the spindle of the first division, and resemble it in lying parallel to the long axis of the cell. Juel advances the theory that the Basidiomycetes are phylogenetically of two groups, "the Protobasidiomycetes (Uredinales, Auriculariales, and Dacryomycetales) and the Autobasidiomycetes (Tremellales and Hymenomycetales)," in the former the spindle lying parallel to the long axis of the basidium, and in the latter at right angles to it.

Maire (36), having re-examined the species (*Auricularia mesenterica*) studied by Juel, states the chromosome number to be two. He terms the bodies figured by Juel protochromosomes, and maintains that two is the constant haploid chromosome number in all the Basidiomycetes (Ishikawa, 23; Tischler, 50). His figures show that after the division of the fusion nucleus in this species no septum is laid down until the spindles of the second division are already formed. A two-celled basidium exists for a very brief period. Since the daughter nuclei of the fusion nucleus do not always divide simultaneously, the two septa last formed are occasionally laid down independently. A case of this kind, in which the basidium contains only two of its three septa, is figured.

None of these investigators describe conjugate divisions in the hyphae. They do not even show that a binucleate condition is a constant characteristic of any definite portion of the life cycle. Their accounts of the nuclear divisions in the basidium are contradictory, and nuclear phenomena following spore germination are not described.

Our knowledge of the nuclear history and general cytology of the higher Hymenomycetes and Gastromycetes is also still far from satisfactory. The basidia in practically all described cases are binucleate in the young condition, and arise from binucleate cells in the subhymenium. Other cells in the hyphae of the sporophore or in the nutritive mycelium may be uninucleate or multinucleate. The accounts of different investigators differ greatly with respect to the point of origin of the binucleate condition in different species. The bulk of the evidence seems to show that the nuclear pairs do not arise at any given point or in any specialized manner. Recently Kniep (28), working with *Corticium varians* Kniep and *C. serum* Pers., has reached the conclusion that the binucleate condition is initiated and maintained by means of clamp connections. He points out also that the basidium is frequently connected by a clamp connection with the cell below, and he presents an interesting argument to show that the basidium is homologous with the ascus. He likens the formation of the clamp connection on the basidium to crozier formation on the ascogenous hypha, and homologizes the terminal cell of the clamp with that of the ascus hook. His work is extremely interesting since it furnishes the most plausible explanation yet advanced of the function of the clamp connections in the Basidiomycetes. His theory fails, however, to explain the large number of described cases

in which the basidia are formed without clamps (Levine, 33). Moreover, it fails to explain how the binucleate condition arises in species lacking clamp connections. He promises to elucidate these latter cases in a further contribution.

It is difficult to apply Kniep's explanation of the origin of the binucleate condition to *Eocronartium muscicola*. If clamp connections occur in this species for a brief period following spore germination, it does not seem logical to suppose that they would function as described by Kniep for a few cell generations and then cease to be developed on all the older mycelium. It seems probable to the writer therefore that clamp connections are wholly absent in *Eocronartium muscicola*.

Since the binucleate series in the Uredinales is initiated by a simple cell fusion, the discovery of a similar phenomenon in members of the Auriculariales would not be unexpected. Possibly such a fusion occurs in *Eocronartium muscicola* at a point in the life cycle following soon after spore germination. Since many facts in connection with this fungus indicate its close relationship with the rust fungi, this is a reasonable hypothesis. The investigation of the cytology of other members of the Auriculariales is very desirable in this connection. It is possible that other species present more favorable material for the determination of the origin of the binucleate condition than is available in *Eocronartium muscicola*. The determination of this point is of unusual interest because of its bearing on the phylogeny of the Uredinales.

## Summary

1. The investigation of the cytology of *Eocronartium muscicola* is based on material from a single host, *Climacium americanum*, collected in the vicinity of Ithaca, N. Y.

2. The mycelium of the parasite is intracellular and permeates throughout the host plant from the underground stolons to the tips of the erect gametophoric branches. All the cells of the mycelium in which the nuclear number has been determined are binucleate, and conjugate divisions occur regularly. Uninucleate or multinucleate cells have not been found.

3. The fungous sporophore arises at the tip of a gametophoric branch of the moss plant, and is formed by the outward growth of the endophytic hyphae. These hyphae pass out into the spaces between

the overlapping moss leaves and grow upward, sheathing the apical region and developing a clavate Typhula-like fruit-body.

4. The cells of the hyphae composing the sporophore are all binucleate.

5. The chromosome number in the conjugate divisions has been determined with reasonable certainty to be four. The nucleolus lies outside the spindle, and enters one of the daughter nuclei in telophase.

6. The young basidia stand at right angles to the surface of the sporophore, and are unicellular and binucleate. Later the pair of nuclei approach each other and fuse.

7. The fusion nucleus passes from the resting into the spirem stage, and later the thread contracts into a definite synaptic knot.

8. The spindle of the first division is intranuclear. It holds no definite position with reference to the long axis of the basidium. A definite centrosome appears at each pole, but no astral radiations have been noted.

9. The chromosome number in the first division is certainly four. As in the conjugate divisions the nucleolus enters one of the daughter nuclei.

10. With the rounding up of the two daughter nuclei the basidium increases greatly in length, and the nuclei migrate into the opposite ends of the cell. They are considerably smaller than the fusion nucleus.

11. The second mitosis is more difficult to study on account of the smaller size of the nuclei, but intranuclear spindles with centrosomes are formed. The two divisions are not always exactly simultaneous in the two nuclei.

12. The four nuclei which round up after the second division migrate apart, and transverse septa are laid down dividing the basidium into four approximately equal superimposed, uninucleate cells. The central septum is laid down first.

13. Each cell of the basidium puts out a long cylindrical sterigma into which passes all the cytoplasm and the nucleus of the basidial cell. The basidium does not produce sterigmata in all its cells simultaneously or in any definite order. The sterigma at maturity is sharp-pointed.

14. A minute, globose spore "initial" forms at the tip of the sterigma, and this develops rapidly into an elongate spore which at maturity is more or less definitely crescent-shaped. All the cytoplasm and the nucleus of the sterigma pass into the spore, the nucleus being

drawn out into a long, irregular, deep-staining rod in order to effect its passage through the narrow canal between the sterigma and the spore. No evidence is given to indicate that the centrosomes function in directing the nucleus into the sterigma or in pulling it into the spore. The elongate rod after its entrance into the spore soon contracts into a globose, homogeneous, deep-staining mass which later takes on the usual appearance of a globose resting nucleus.

15. The spore is liberated from the basidium in the uninucleate condition. Germination in wet weather frequently occurs at once on the sporophore. The spores may be induced to germinate in nutrient solutions and on solid media. In germination the cytoplasm and nucleus pass out into a germ-tube, but the nucleus never undergoes division there. There is no increase in the amount of cytoplasm, and septa are not formed.

16. Nothing is known of that phase of the nuclear history which follows spore germination and precedes the appearance of the binucleated series of cells in the endophytic hyphae. Consequently the origin of the binucleate condition has not been determined. The difficulties which have been encountered in the investigation of this phase of the life cycle are discussed in the writer's previous paper on *Eocronartium muscicola* (12).

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## EXPLANATION OF PLATES XXX–XXXII

All the figures were drawn with the aid of a camera lucida. A Zeiss 2 mm. apochromatic oil immersion objective (1.4 N.A.) and an 8 compensating ocular were used. As reproduced the figures represent a magnification of about 2150.

FIGS. 1–4. Endophytic hyphae of *Eocronartium muscicola* in the tissue of *Climacium americanum*. The host cells are merely outlined.

FIG. 1. A fungous hypha showing a single extremely long binucleate cell. The deep-staining bodies at the upper end are cytoplasmic granules. The deep-staining pads on the transverse septa in this and other figures probably indicate the presence of protoplasmic connections. The large, much-elongated host cells are characteristic of the main axis of the moss gametophore.

FIG. 2. A much shorter cell with more minute nuclei; in the same general region of the host.

FIG. 3. Terminal cell of a hypha passing from the interior of a gametophoric branch out into the space beneath one of the leaves. The leaf is shown in section at the right. The tip of the hypha has turned upward toward the apex of the host branch where later it would unite with other hyphae to form the sporophore.

FIG. 4. Terminal cell of a hypha in the apical region of the host.

FIGS. 5–19. Hyphae in the interior of the sporophore.

FIG. 5. Unusually short cells with minute nuclei. The cells are binucleate and the pads on the septa are prominent.

FIG. 6. A very long cell in an adjacent hypha. The cell contains two large nuclei, and numerous deep-staining cytoplasmic granules.

FIG. 7. A pair of nuclei in a cell of one of the hyphae of the sporophore. They are in the spirem condition, possibly near synapsis.

FIG. 8. Another pair of nuclei much smaller in size and possibly in very early prophase. Each contains four deep-staining bodies resembling chromosomes.

FIGS. 9–16. Other pairs of nuclei in various stages of conjugate division.

FIGS. 17–18. Four-nucleate cells; the two pairs of nuclei in each cell resulting from conjugate division.

FIG. 19. A terminal binucleate cell on a hypha at the periphery of the sporophore. Such a cell by division cuts off the young binucleate basidium.

FIGS. 20–45. Basidia.

FIGS. 20–23. Young binucleate basidia. There is considerable variation in shape.

FIG. 24. A young basidium in which nuclear fusion is taking place.

FIG. 25. A basidium in which the fusion nucleus still contains two nucleoli.

FIG. 26. Fusion nucleus at a stage near the resting condition.

FIG. 27. Fusion nucleus at a slightly later stage showing chromatin aggregated into larger masses at the interstices of the network.

FIG. 28. Spirem thrown into eight definitely thickened loops.

FIG. 29. Spirem loops less definite.

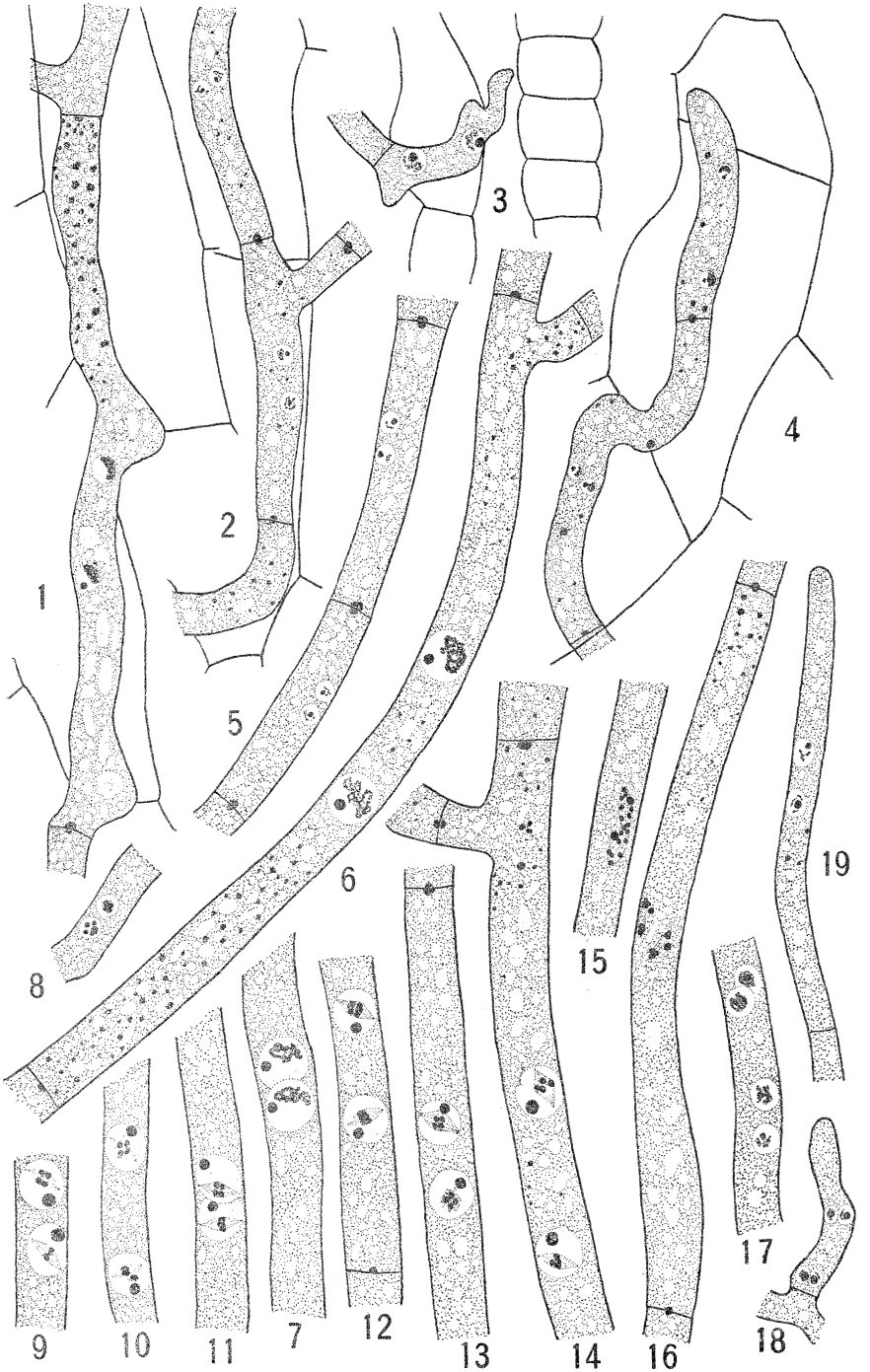
FIGS. 30, 31. Spirem at one side of nucleus, preceding synapsis.

FIG. 32. Synapsis; nucleolus enmeshed in the chromatin strand.

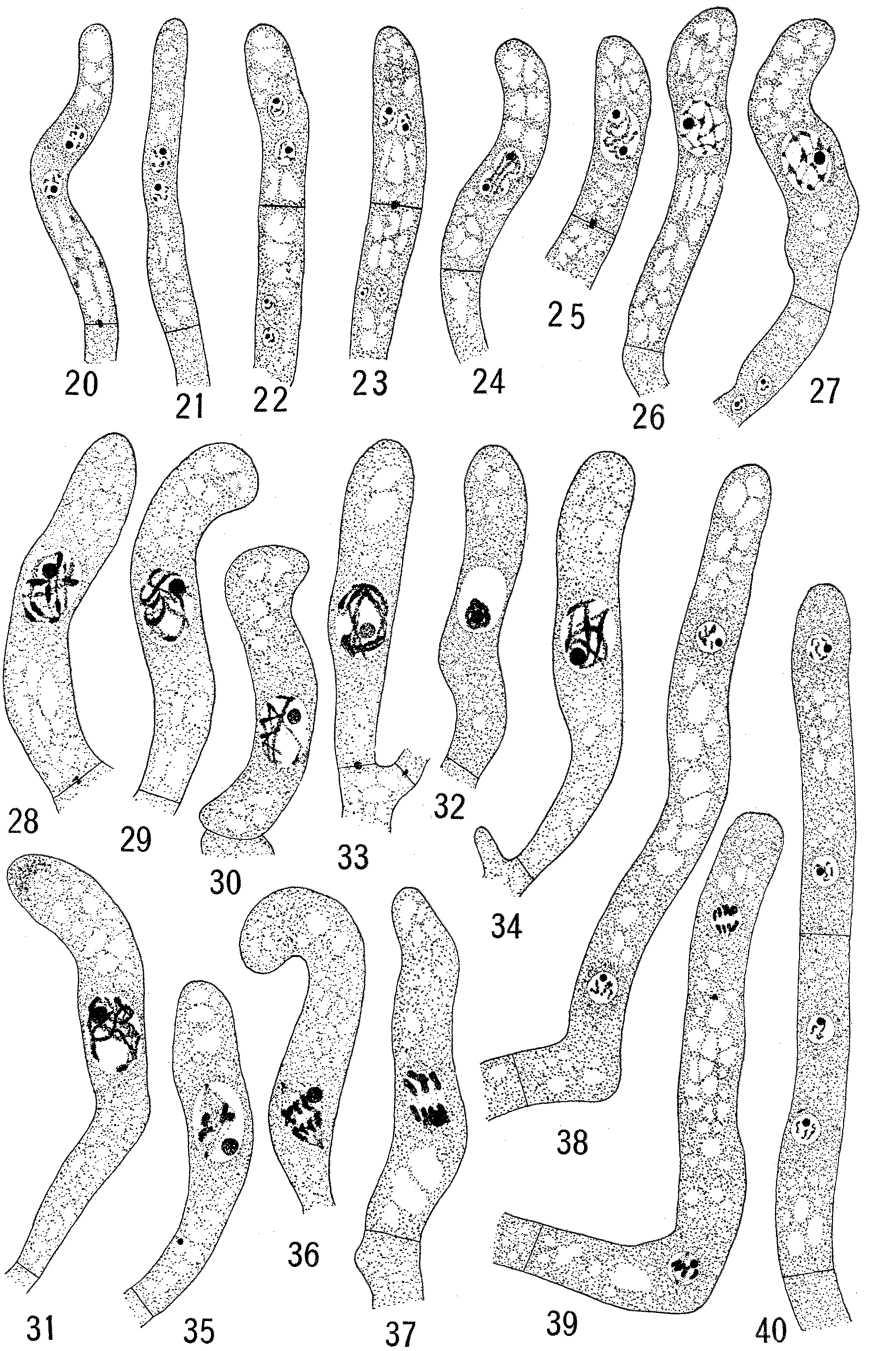
FIG. 33. A spirem giving some indication of a longitudinal split.

FIG. 34. A segmented spirem.

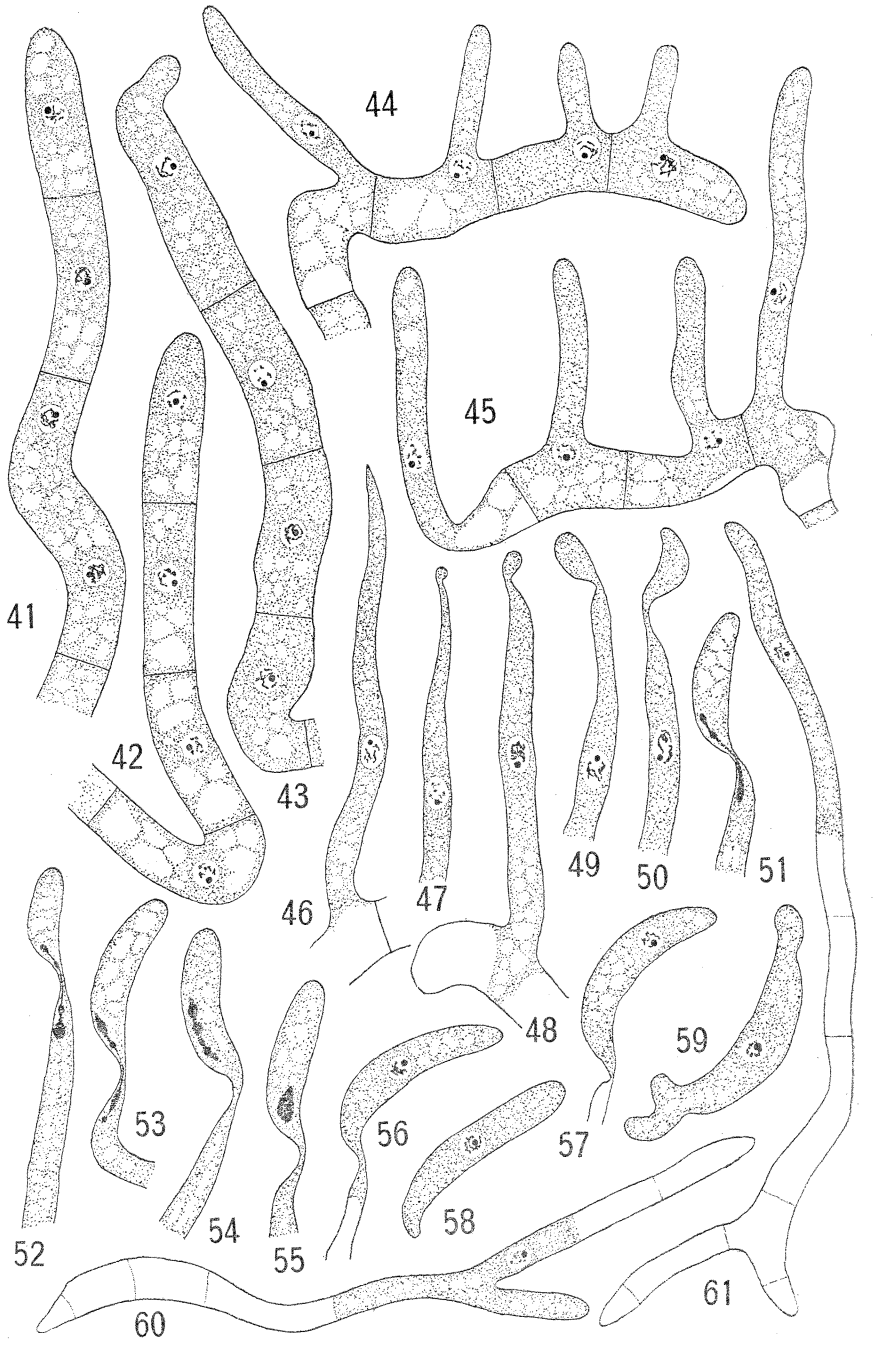
FIG. 35. First nuclear division showing four chromosomes; metaphase.




FITZPATRICK: CYTOLOGY OF EOCRONARTIUM.




FITZPATRICK: CYTOLOGY OF EOCRONARTIUM.




FITZPATRICK: CYTOLOGY OF EOCRONARTIUM.

FIG. 36. The chromosomes moving toward the poles; anaphase.

FIG. 37. Telophase: nucleolus at one pole.

FIG. 38. An elongated, binucleate basidium.

FIG. 39. Second nuclear division, one nucleus dividing more rapidly than the other.

FIG. 40. A two-celled, four-nucleate basidium. The middle septum is always the first formed.

FIG. 41. A three-celled basidium. The second mitosis in this basidium was evidently not exactly simultaneous in the two nuclei. Consequently one septum is partially formed while the other has not yet appeared.

FIG. 42. A typical four-celled basidium.

FIG. 43. A mature four-celled basidium which is beginning to form a sterigma from the apical cell.

FIGS. 44, 45. Mature basidia forming sterigmata.

FIGS. 46–58. Sterigmata and spore formation.

FIG. 46. A completely formed sterigma with an acuminate tip.

FIG. 47. Sterigma at the tip of which the spore "initial" is beginning to form.

FIGS. 48–50. Stages in the enlargement of the young spore. The sterigma in each case contains a normal nucleus.

FIGS. 51–53. The passage of the nucleus into the spore. It is drawn out into a long, irregularly beaded rod.

FIGS. 54–55. Stages in the transformation of the nucleus from the deep-staining rod into its normal form.

FIG. 56. Normal nucleus in a nearly mature spore.

FIG. 57. Mature spore attached to the sterigma.

FIG. 58. Mature, detached spore.

FIGS. 59–61. Spore-germination.

FIG. 59. Early stage in spore germination; germ-tubes formed at both ends of the spore. This spore germinated on the sporophore in wet weather.

FIGS. 60–61. Spores induced to germinate in hanging drop culture. The apparent septa are merely dried hyaloplasm.